NADPH IMPROVES RED COLOR FORMATION IN BEEF INCUBATED WITH NO-SYNTHASE AND L-ARGININE.

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I. INTRODUCTION

Numerous methods have been proposed to reduce nitrite levels in meat products or find nitrite alternatives (1). Our proposition involves utilizing the enzyme Nitric oxide (NO) synthase, naturally present in living cells, and crucial in various physiological processes including blood pressure regulation, immune response, and neuronal signaling. NO synthase catalyzes the conversion of arginine into nitric oxide under specific conditions, dependent on the isoform of the enzyme: inducible (iNOS), endothelial, or neuronal (2). This study extends our previous research and aims to evaluate the effects of varying time, enzyme, and arginine concentrations. NADPH, the reduced form of nicotinamide adenine dinucleotide phosphate serves as a cofactor for iNOS. Given its ubiquitous presence in mammalian cells, (in the initial phase of the experiment) we anticipated its existence in the meat used for our experiment. In this phase of of our research, we investigated changes in meat color in the presence or absence of NADPH.

II. MATERIALS AND METHODS

Beef *m. semimembranosus* from a local retailer (Makro, Poland) was ground (3 mm plate) (Mado, Germany). The whole mass was mixed, divided into 50 g portions, and placed in glass beakers along with enzyme (iNOS- 1U = 254mg of protein), L-arginine and/or NADPH (Table 1).

Table 1. Additives used in the test samples with NADPH (per 50g portion)

Sample ID	iNOS [U]	L-Arginine [g]	NADPH [g]	Time [h]	Sample ID	iNOS [U]	L-Arginine [g]	NADPH [g]	Time [h]
1	0.5	0.05	0.06	3h	12	0.5	0.05	0.06	6h
2	0.5	0.25	0.06		13	0.5	0.25	0.06	
3	0.5	0.75	0.06		14	0.5	0.75	0.06	
4	1.0	0.05	0.12		15	1.0	0.05	0.12	
5	1.0	0.25	0.12		16	1.0	0.25	0.12	
6	1.0	0.75	0.12		17	1.0	0.75	0.12	
7	1.5	0.05	0.18		18	1.5	0.05	0.18	
8	1.5	0.25	0.18		19	1.5	0.25	0.18	
9	1.5	0.75	0.18		20	1.5	0.75	0.18	
10	No additives				21	No additives			
11	4 mL $NaNO_2$ (1g/L)				22	4 mL $NaNO_2$ (1g/L)			

Meat was incubated at 37 °C for 3 h or 6 h and cooked afterwards (95°C/30 minutes), cooled down and stored (4°C). Color of the samples was measured (Konica Minolta CM-3500d colorimeter, Osaka, Japan) (measurement mode: D/8 (SCE), D65 illuminate,10° viewing angle. L*, a*, and b* coordinates were measured. Redness (a*/b*) and Chroma (C= $[a^{*2}+v^{*2}]^{0.5}$) were calculated. The results were subjected to ANOVA analysis, the differences were tested using Tukey test at $P<0.05$.

III. RESULTS AND DISCUSSION

All samples containing 1.5% L-Arginine showed notably higher a* values (Table 2) and redness (a/b) values compared to samples with lower L-Arginine concentrations. This supports the findings of Bludau et al.,(3) who investigated beef frankfurters with L-Arginine addition and achieved satisfactory results, albeit lower than nitrite-cured sausages. Addition of NADPH to all samples resulted in increased redness, although statistically significant differences were only observed for samples incubated for 3 hours with 1U of NO synthase (samples 4, 5, and 6), or with 1.5 U of NO synthase

and 1.5% L-Arginine, as well as for samples incubated for 6 hours with either 1 or 1.5U of NO synthase and 1.5% L-Arginine. These findings suggest that NADPH may enhance NO synthase activity, particularly at higher enzyme and L-Arginine concentrations, when sufficient iNOS and its substrate are available. Samples 6, 20, 17, 3, and 9 exhibited the highest redness values, although these values were only around 60% of those obtained for nitrite-cured samples.

Table 2. CIELab parameters of meat samples incubated with or without NADPH (mean values ± standard errors).

Sample ID	L*(D65)		a*(D65)		b*(D65)		a*/b*	
	no NADPH	NADPH	no NADPH	NADPH	no NADPH	NADPH	no NADPH	NADPH
1	46.1 ±0.7	45.3 ±1.0	6.4 ±0.1	6.8 ±0.1	15.3 ±0.2	14.6 ±0.2	0.4 ±0.0	0.5 ±0.0
2	46.3 ±0.8	44.8 ±0.9	6.9 ±0.1	7.3 ±0.1	15.0 ±0.2	14.1 ±0.3	0.5 ±0.0	0.5 ±0.0
3	42.4 ±0.6	43.3 ±0.7	7.6 ±0.1	8.6 ±0.1	13.0 ±0.3	13.7 ±0.2	0.6 ±0.0	0.6 ±0.0
4	48.0 ±0.5	44.7 ±0.9	6.1 ±0.1	6.9 ±0.2	14.7 ±0.2	14.0 ±0.3	0.4 ±0.0	0.5 ±0.0
5	45.8 ±0.9	45.3 ±0.8	7.0 ±0.1	7.3 ±0.1	15.1 ±0.2	14.1 ±0.3	0.5 ±0.0	0.5 ±0.0
6	42.6 ±0.7	41.5 ±0.6	7.6 ±0.1	8.3 ±0.2	13.2 ±0.2	12.0 ±0.2	0.6 ±0.0	0.7 ±0.0
7	45.9 ±1.2	47.0 ±0.4	6.3 ±0.2	6.5 ±0.1	14.8 ±0.3	14.3 ±0.1	0.4 ±0.0	0.5 ±0.0
8	46.5 ±0.6	45.6 ±0.4	6.4 ±0.2	7.0 ±0.1	14.5 ±0.2	14.7 ±0.2	0.4 ±0.0	0.5 ±0.0
9	44.0 ±0.4	43.4 ±0.4	7.2 ±0.1	8.2 ±0.1	13.6 ±0.3	13.3 ±0.4	0.5 ±0.0	0.6 ±0.0
10	46.3 ±0.9	43.6 ±0.9	6.1 ±0.1	6.4 ±0.1	14.6 ±0.3	14.1 ±0.4	0.4 ±0.0	0.5 ±0.0
11	43.1 ±0.7	45.6 ±0.6	13.6 ±0.3	13.9 ±0.2	12.6 ±0.1	12.4 ±0.1	1.1 ±0.0	1.1 ±0.0
12	**47.8 ±0.8**	**50.2 ±0.6**	**6.3 ±0.1**	**6.1 ±0.1**	**15.2 ±0.2**	**14.6 ±0.2**	**0.4 ±0.0**	**0.4 ±0.0**
13	45.8 ±0.9	47.8 ±0.7	6.6 ±0.2	6.7 ±0.1	15.2 ±0.2	14.0 ±0.2	0.4 ±0.0	0.5 ±0.0
14	42.4 ±0.6	43.8 ±0.3	7.6 ±0.1	7.8 ±0.1	13.1 ±0.3	13.3 ±0.2	0.6 ±0.0	0.6 ±0.0
15	46.6 ±1.2	48.6 ±1.0	6.8 ±0.3	5.8 ±0.1	15.7 ±0.3	13.8 ±0.3	0.4 ±0.0	0.4 ±0.0
16	46.8 ±0.6	48.9 ±0.4	6.7 ±0.1	6.4 ±0.1	15.1 ±0.2	13.7 ±0.3	0.4 ±0.0	0.5 ±0.0
17	43.1 ±0.7	43.4 ±0.6	7.2 ±0.1	7.6 ±0.2	13.0 ±0.4	12.1 ±0.3	0.6 ±0.0	0.6 ±0.0
18	47.9 ±0.5	49.7 ±0.5	6.0 ±0.1	6.0 ±0.1	15.3 ±0.1	14.1 ±0.2	0.4 ±0.0	0.4 ±0.0
19	46.4 ±0.5	48.6 ±0.5	6.7 ±0.2	6.6 ±0.1	15.5 ±0.1	14.4 ±0.2	0.4 ±0.0	0.5 ±0.0
20	42.2 ±0.5	44.1 ±0.8	7.4 ±0.1	7.8 ±0.1	13.3 ±0.2	12.1 ±0.3	0.6 ±0.0	0.7 ±0.0
21	48.9 ±0.1	48.2 ±0.8	5.7 ±0.1	5.6 ±0.1	15.1 ±0.1	13.9 ±0.3	0.4 ±0.0	0.4 ±0.0
22	**45.6 ±0.5**	**47.2 ±0.7**	**13.7 ±0.2**	**12.8 ±0.3**	**12.6 ±0.01**	**11.4 ±0.2**	**1.1 ±0.0**	**1.1 ±0.0**

pH of the samples ranged from 5.9 (11,12, 21, 22) to 6.8-7.0 (3, 6, 9, 14, 17, 20). pH increase is usually associated with higher redness (4).Since these samples also had higher pH compared to the others, it raises the question if the improved redness can be solely attributed to the higher pH.

IV. CONCLUSION

NADPH enhances the color of meat when higher levels of iNOS (1.0 and 1.5U) and L-Arginine (1.5g/100g of meat) are present. The a/b values saw an increase of 12.3-19.8% compared to samples without NADPH. However, these values were only about 60% of those in cured samples.

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REFERENCES

1.Zhang Y., Zhang Y., Jia J., Peng H., Qian Q., Pan Z., Liu D. (2023) Nitrite and nitrate in meat processing: Functions and alternatives. Current Research in Food Science. 2023;6:100470.
2.Förstermann U., Sessa W..C. (2012). Nitric oxide synthases: regulation and function. European heart journal. 33(7):829-37.
3.Bludau S.E., Modrow K.M., Osburn W.N., (2022) Evaluation of an amino acid based alternative curing system on the physiochemical and shelf life attributes of beef frankfurters. Abstracts from 2021 AMSA Reciprocal Meat Conference. Meat and Muscle Biology 6(2) 13203.
4.King D.A., Hunt M.C., Barbut S., Claus J.R., Cornforth D.P., Joseph P,. Kim B., Lindahl G., Mancini R., Nair M. . (2023). American Meat Science Association guidelines for meat color measurement. Meat and Muscle Biology 6(4).